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Achromobacter sp. and *Virgibacillus pantothenticus* as models of thermo-tolerant lipase-producing marine bacteria from North Delta sediments (Egypt)

Hanan Abd-Elnaby, Ehab A. Beltagy*, Gehan M. Abo-Elela and Nermeen A. El-Sersy

National Institute of Oceanography and Fisheries, Microbiology Laboratory, Marine Environment Division, Alexandria, Egypt.

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Sediments of North Delta (Egypt) are a rich source of many thermo-tolerant bacteria, which could be used as a good source of many enzymes. Sediment samples were collected from six different sites in North Delta region. The counts of thermo-tolerant marine bacteria (at 55°C) in sediment samples ranged from $9.8x10^2$ to $6.8x10^3$ CFU g^{-1}. It was found that the occurrence of thermo-tolerant bacteria in North Delta sediments at 55°C reached 11.1% with respect to the total viable count at 30°C. Five of seven selected thermo-tolerant bacterial isolates showed optimum growth at pH 9 and NaCl concentration of 4% (w/v) after 24 h of incubation at 55°C. Two thermo-tolerant marine bacteria were selected and identified as *Achromobacter* sp. HEGN 014 and *Virgibacillus pantothenticus* HEGN 114 using 16S rDNA analysis. Their sequence similarities were 96 and 97%, respectively. Their optimum growth was at pH 9 and NaCl concentration of 4% (w/v) with tolerance to higher concentration of 7 and 9% (w/v), respectively. Moreover, both strains were investigated to produce an extracellular lipase, while they showed no productivity for cellulase, chitinase or protease at 55°C, however, gelatinase was produced only by *V. pantothenticus* HEGN 114. Antibiotics resistance was observed for *Achromobacter* sp. HEGN 014 and *V. pantothenticus* HEGN 114. The optimum reaction temperatures for the purified lipases from both strains were the same at 35 and 55°C. The amino acid analysis showed that arginine represented 89.982 mmole of total detected amino acids of lipase produced from *Achromobacter* sp. HEGN 014 with tolerance to higher concentration of 7 and 9% (w/v) NaCl.

Key words: *Achromobacter* sp., *Virgibacillus pantothenticus*, thermo-tolerant, lipase.

INTRODUCTION

Life exists almost everywhere on earth. Presence of liquid water is a prerequisite for life (Oren, 2008). Moderate environments are important to sustain life. Any environmental condition that can be perceived as beyond the normal acceptable range is an extreme condition. A variety of microbes, however, survives and grows in such environments. These organisms, known as extremophiles, not only tolerate specific extreme

*Corresponding author. E-mail: ehab.beltagy@gmail.com. Tel: 002 01005243809.

condition(s), but also usually require these for survival and growth. Most extremophiles are found in the microbial world. The range of environmental extremes tolerated by microbes is much broader than other life forms (Satyanarayana et al., 2005). In these habitats, environmental conditions such as pH, temperature and salinity concentrations are extremely high or low. Extreme environments are populated by groups of organisms that are specifically adapted to these particular conditions (Mahmoud, 2006).

As a result of adaptation to extreme environments, extremophiles have evolved unique properties, which can be of biotechnological and commercial significance (Margesin and Schinner, 2001). Life in extreme environments has been studied intensively focusing attention on the diversity of organisms, molecular and regulatory mechanisms involved. The products obtainable from extremophiles such as proteins, enzymes (extremozymes) and compatible solutes are of great interest to biotechnologists (Satyanarayana et al., 2005).

Despite the fact that, to date more than 3000 different enzymes have been identified and many of these have found their way into biotechnological and industrial applications, the present enzymes toolbox are still not sufficient to meet all demands.

A major cause for this is the fact that many available enzymes do not withstand industrial reaction conditions (Madigan and Marrs, 1997). The major share of the industrial enzyme market has been occupied by hydrolytic enzymes such as lipases, esterases, proteases and amylases (Gupta et al., 2004).

Lipids constitute a large part of the earth's biomass and lipolytic enzymes play an important role. Lipases and esterase have been recognized as very useful biocatalyst (Mohan et al., 2008). Lipases are a class of hydrolases that catalyze a variety of reactions, such as the hydrolysis of fatty acid ester, trans-esterification and ester synthesis at the interface between the insoluble substrate and water.

Microbial lipases are currently receiving much attention because of their biotechnological potential applications (Aly et al., 2012; Wang et al., 2014). They are the major industrial enzymes extensively used in pharmaceuticals, textiles, food, medical, detergent manufacturing and other chemical industries (Mohan et al., 2008; Sangeetha et al., 2011; Aly et al., 2012; Nerurkar et al., 2013; Chatterjee et al., 2014).

The major cause of limiting industrial usage of known lipases or esterases is their limited thermo-stability, mainly at high temperatures, pH and inorganic salts. Therefore, the search for new microbial enzyme sources is important for the development of new thermo-stable enzymes for industrial applications (Gupta et al., 2004; Faiz et al., 2007).

This study aims to isolate and characterize thermo-tolerant marine bacteria from North Delta sediments. Moreover, the study extended to screen their abilities to produce industrially valuable enzymes.

MATERIALS AND METHODS

Sampling and isolation of bacteria

Sediment samples were collected from six different sites (Demitta, Gamasa, Baltim, El-Brolus, Abou-Kashaba and Rashid) in North Delta region. For estimation of total viable count (TVC), sediments samples were suspended in 100 ml sterilized aged seawater to dissociate the adhered bacterial population, then diluted up to 10^{-6}. Each dilution was plated by pour plat method onto seawater nutrient agar (SWNA) with the following composition g 1^{-1}: peptone, 5.0; yeast extract, 3.0; agar, 15; aged seawater. Plates were incubated at 30 and 55°C for 24-48 h. Triplicates were used for each sample.

Isolates selection

Seven bacterial isolates have been isolated from growing colonies at 55°C. These colonies were selected according to morphological characters to represent the most dominant colonies. These seven thermo-tolerant bacterial isolates were selected for further studies: Demitta (A1), Gamasa (A2), Baltim (A4), El-Brolus (A9 and A10), Abou-Kashaba (A22) and Rashid (A31).

Testing for thermotolerance, alkalitolerance and halotolerance

All growth experiments were conducted in triplicate in seawater nutrient broth medium, unless otherwise stated. Growth was determined by measuring optical density at 550 nm ($O.D._{550}$) with a spectrophotometer (U–1500, Hitachi). Effect of temperatures on growth was determined in the range of 35 - 85°C. Effect of pH on growth was determined by adjusting pH of the medium from 5 to11. NaCl requirements were determined in the same medium containing 4-13% (w/v) NaCl.

Molecular identification

The identification was carried out at City for Scientific Research and Technology Applications, Arid Land Institute, Molecular Plant Pathology Department, New Borg El Arab City, 21934, Alexandria, Egypt.

Characterization of the selected strains

The characters of the selected organisms were studied following the standard microbiological methods as described in Bergy's manual (Holt et al., 1994). Colony morphology, Gram reaction and spore characteristics were observed. The physiological and biochemical characters included production of β- galactosidase, arginine dehydrolase, lysine decarboxylase, ornithine decarboxylase, sulphide, urease, tryptophane deaminase, indole, acetoin and gelatinase, oxidase, catalase and citritase tested. Also, the utilization of different sugars such as D-glucose, D-mannitol, inositol, D-sorbitol, rhamnose, D-sucrose, D-melibiose, amygdalin and L- arabinose were determined.

Resistance to antibiotics

Resistance of thermo-tolerant strains were tested against: ampicillin-sulbactam, 20 µg; ciprocin, 5 µg; gentamycin, 10 µg;

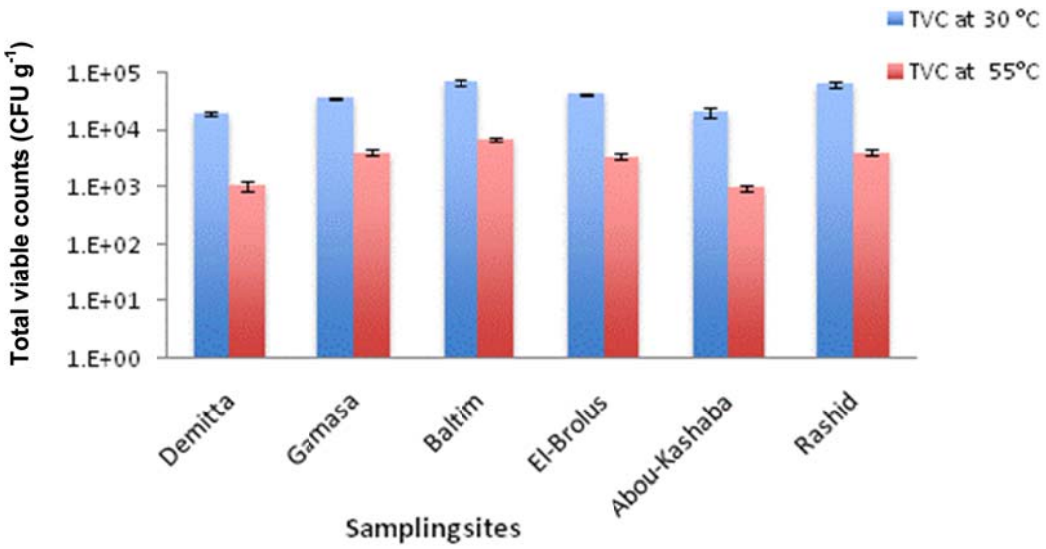

Figure 1. The viable counts of thermotolerant bacteria (CFU g^{-1}) in North Delta sediments (values are average ± SD).

ampicillin, 10 µg; imipenem, 10 µg; norfloxacin, 10 µg; cephalexin, 30 µg; cefadroxil, 30 µg; ceftazidine, 30 µg; erythromycin, 15 µg; and flucloxacillin, 5 µg by disk diffusion method (Bauer et al., 1966).

Enzymes productivity

The selected isolates were tested for production of gelatinase (Ventosa et al., 1982), protease, lipase, chitinase (Hankin and Anagnostakis, 1975) and cellulase (Ariffin et al., 2006) enzymes. After incubation at 37°C, the appearance of clear zones around the colonies was scored as a positive result.

Lipase production

Mineral based broth contained (g l^{-1}): $NaNO_3$, 0.30; K_2HPO_4, 0.01; $MgSO_4.7H_2O$, 0.05; KCl, 0.05; $FeSO_4.7H_2O$, 0.001; yeast extract, 0.50 and sucrose, 0.5. The cotton seeds oil (100 ml) were warmed to 70°C, then emulsified with Arabic gum (0.5 g) with continuous stirring, the pH of the medium was adjusted to 7.5. For lipase production, mineral bases broth was supplemented by 1% (v/v) cotton seeds oil, and production media (50 ml each in 200 ml capacity Elementary flasks) inoculated by 48 h old culture. The cells free broth was harvested by centrifugation at 10,000g and 4°C (Kanwar et al., 2006).

Lipase assay

Lipase activity was measured by titrimetric method using cottonseeds oil as a substrate. Cottonseeds oil (10% v/v) was emulsified with Arabic gum (5% w/v) in 0.05 mM Tris buffer pH 7.0. 100 µl of enzyme was added to the emulsion and incubated for 30 min at 37°C. The reaction was stopped and fatty acids were extracted by addition of 1.0 ml of acetone : ethanol solution (1:1). The amount of the fatty acids liberated was estimated by titrating with 0.05 M NaOH until pH was 10.5 using a phenophathelin indicator (Jensen, 1983). One unit of enzyme is defined as the amount of enzyme required to hydrolyse µmol of fatty acids from triglycerides per minute.

Effect of different temperatures on enzyme activity

To examine the effect of temperature of the reaction on the activity of the purified enzyme, the enzymatic reaction was carried out for 30 min at different temperature: 35, 40, 45, 50, 55 and 60°C using an enzyme protein and substrate concentration of 4 mg and 0.1 ml cottonseeds oil, respectively, per 1200 µl reaction mixture. A control was made using previously heated enzyme solution in the reaction.

Amino acids analysis

Analysis of amino acids was conducted in central laboratories unit, National Institute of Oceanography and Fisheries (NIOF) using AAA-Direct, Dionex Amino Analyzer and Amino Pac PA10 according to Irvine (1997).

RESULTS

Viable count of thermotolerant bacteria

The occurrence of thermo-tolerant bacteria in North Delta sediments reached 11.1% of the total isolated bacteria in Gamasa. The highest count of thermo-tolerant marine bacteria (at 55°C) in sediment samples was detected in Baltim ($6.8x10^3$ ± 4.9 $x10^2$ CFU g^{-1}) which represented 10.13% of the total viable count at 30°C. The lowest count was estimated in Abou-Kashaba ($9.8x10^2$ ± 1.1 $x10^2$ CFU g^{-1}) representing 4.67% of the total viable count at 30°C (Figure 1).

Growth of isolates at different temperatures

The growth (OD_{550}) of the seven selected isolates at different temperatures was determined after 24 h of incubation in the standard medium. All isolates were able to grow over a wide range of temperature from 35 to

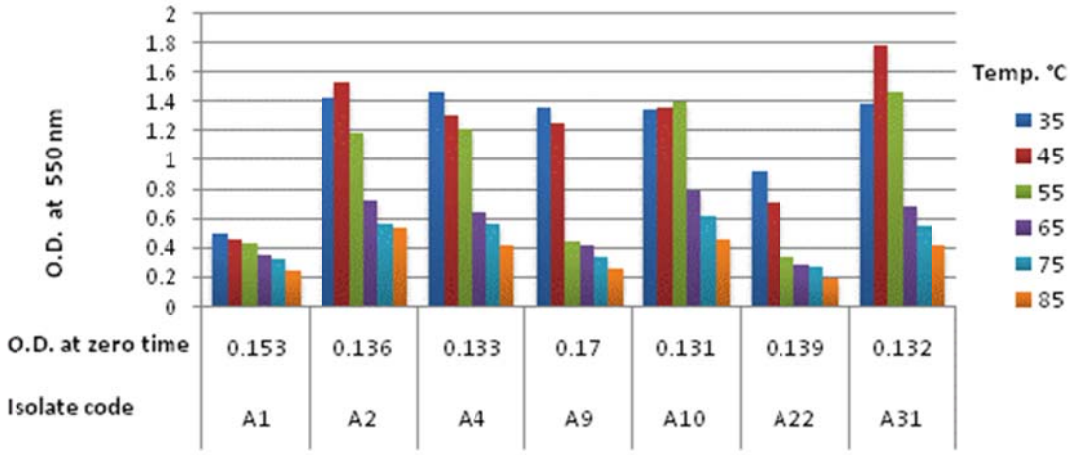

Figure 2. The growth ($O.D._{550}$) of the selected isolates at different temperatures after 24 h incubation.

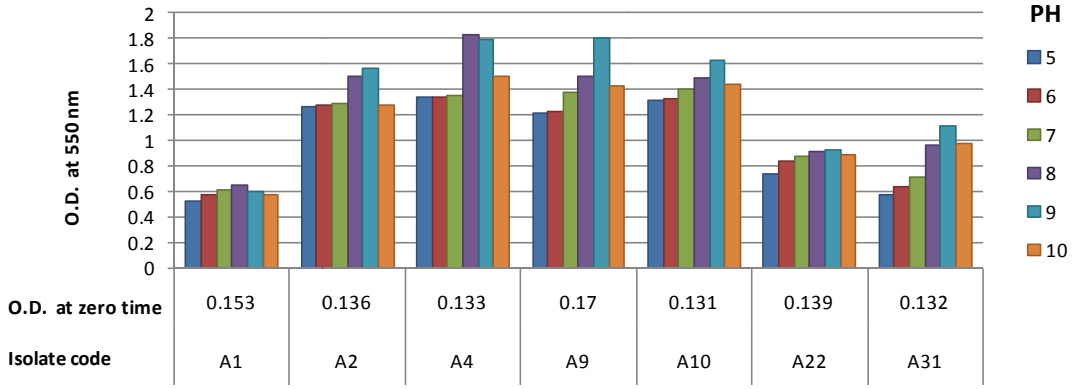

Figure 3. The growth ($O.D._{550}$) of the selected isolates at different pH after 24 h incubation.

85°C (Figure 2). Optimum growth of isolates A_1, A_4, A_9 and A_{22} were at 35°C, while the optimum growth of isolates A_2 and A_{31} were at 45°C. The optimum growth of isolate A_{10} was at 55°C. Isolates A_{10} and A_{31} showed the maximum tolerance to the elevated temperature.

Growth of the isolates at different pH

In order to test the effect of pH on the growth, the interested isolates were subjected to different pH. All isolates were able to grow at wide range of pH after 24 h of incubation at 55°C (Figure 3). Optimum growth of isolates A_1 and A_4 were at pH 8, but the optimum growth of isolates A_2, A_9, A_{10}, A_{22} and A_{31} were estimated at pH 9.

Growth of the isolates at different concentrations of sodium chloride

Increasing the NaCl concentration in the medium showed halotolerance. The isolates showed wide range of salinity tolerance (Figure 4). All isolates gave the maximum growth at salinity 4% (w/v) after 24 h of incubation at 55°C. Considerable growth was determined at 7% NaCl for isolate A_{10}, and at 9% NaCl for isolate A_{31}. Isolate A_{31} showed considerable growth at higher ranges. Isolate A_{31} showed the maximum tolerance to 13% NaCl.

Molecular identification of the selected isolates

DNA of the selected isolates was extracted and the 16S rDNA gene fragment was amplified for partial sequence. The produced amplicons were analyzed using agarose gel electrophoresis. The GenBank accession number for the 16S rDNA sequences were KP212417 and KP212418 for isolates A_{10} and A_{31} respectively. They were identified as *Achromobacter* sp. HEGN 014 and *Virgibacillus pantothenticus* HEGN 114 with similarity percentage 94 and 96% respectively. Figures 5 and 6 represents the phylogenetic relationships among representative

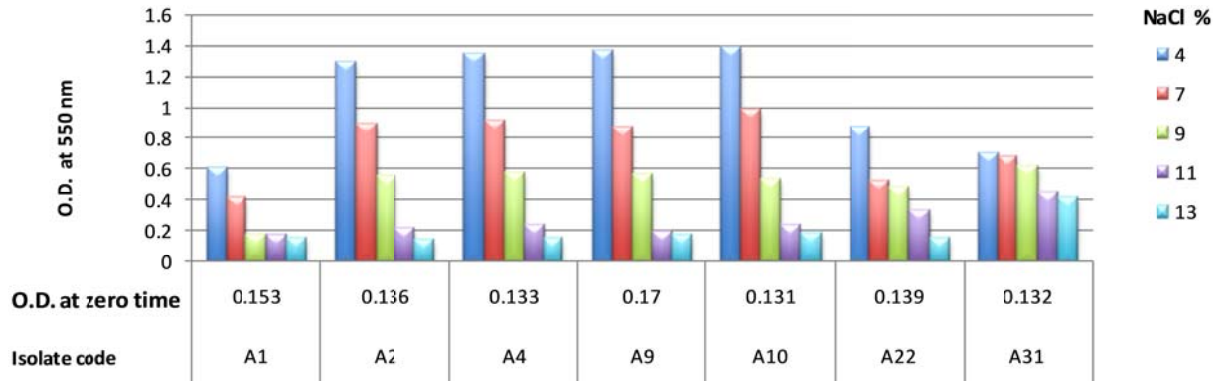

Figure 4. The growth ($O.D._{550}$) of the selected isolates at different salinities (NaCl % w/v) after 24 h incubation.

Figure 5. Phylogenetic relationships among representative experimental strain and the most closely related *Achromobacter* sp. HEGN 014.

experimental strains and the most closely related species using FAST MINIMUM EVOLUTION TREE METHOD, NCBI web server.

Characterization of the strains

The physiological and biochemical characters of *Achromobacter* sp. HEGN 014 (the isolate code A10) and *Virgibacillus pantothenticus* HEGN 114 (the isolate code A31) are represented in Table 1.

Resistance to antibiotics

The thermo-tolerant strains were characterized by resistance to most of the tested antibiotics (Table 2). It

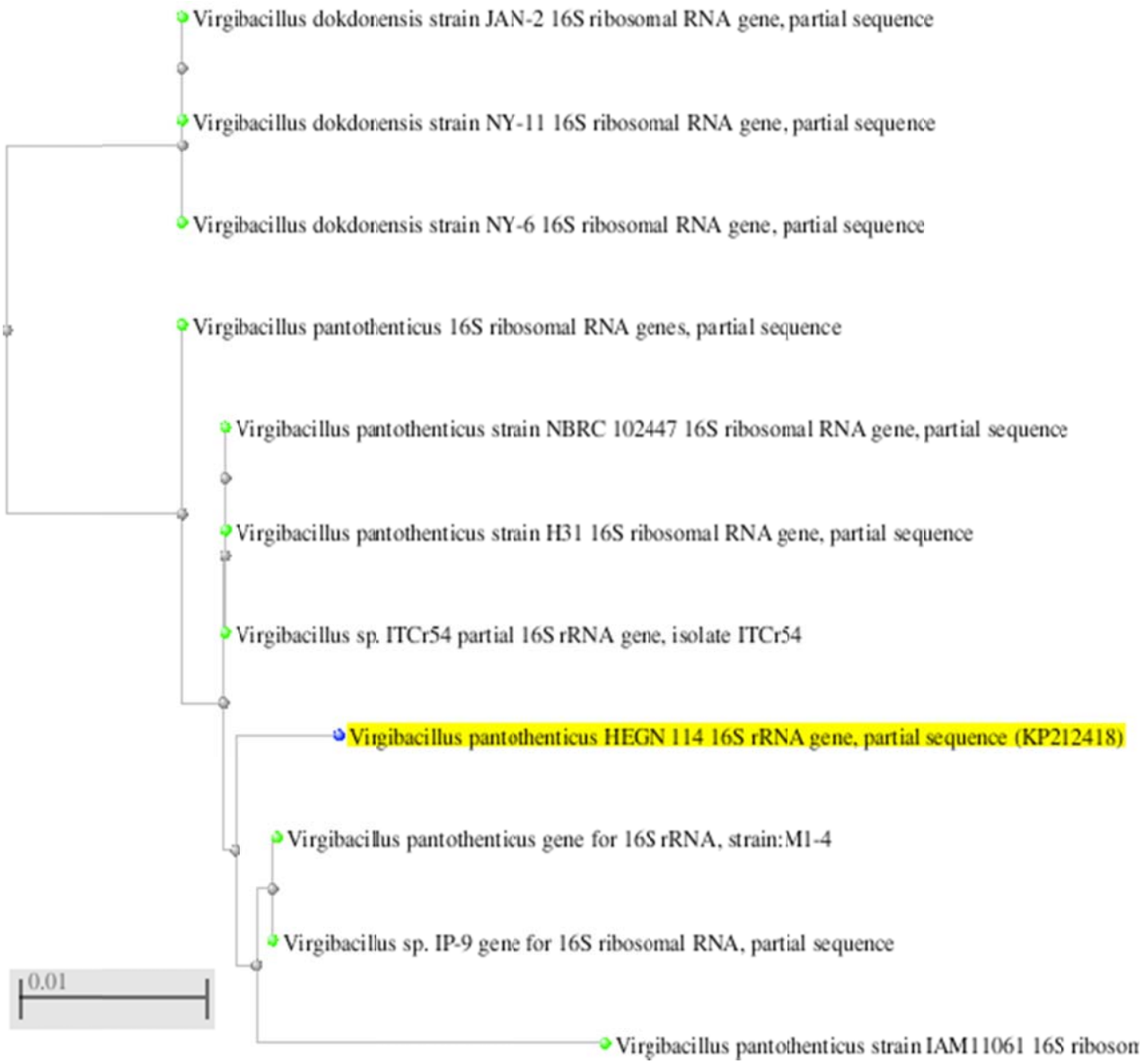

Figure 6. Phylogenetic relationships among representative experimental strain and the most closely related *V. pantothenticus* HEGN 114.

was noticed that *Achromobacter* sp. HEGN 014 resisted most tested antibiotics, gentamycin, 10 µg; ampicillin, 10 µg; imipenem,10 µg; norfloxacin,10 µg; cephalexin, 30 µg; cefadroxil, 30 µg; ceftazidine, 30 µg; erythromycin, 15 µg; and flucloxacillin, 5 µg except ampicillin-sulbactam (20 µg) and ciprocin (5 µg) where the detected inhibition zones were 20 and 30 mm, respectively. On the other side, *V. pantothenticus* HEGN 114 resisted all tested antibiotics.

Enzymes productivity

Thermo-tolerant *Achromobacter* sp. HEGN 014 and *V. pantothenticus* HEGN 114 showed good activities for lipase production, but gelatinase was produced only by *V. pantothenticus* HEGN 114. Both strains showed no activity for protease, chitinase, or cellulase productivity at 55°C (Table 3). The more width of the clearing zone around the strain growth indicates more activity. This indicate that the degradation zone which resulted from the growth of *Achromobacter* sp. HEGN 014 (++) was larger than that produced by *V. pantothenticus* HEGN 114 (+).

Effect of different temperatures on lipase activity

Lipase enzyme produced from selected strains was partially purified using ammonium sulphate precipitation

Table 1. The characteristics of the interested strains.

Characteristic	*Achromobacter* Sp*	*Virgibacillus pantothenticus***	Characteristic	*Achromobacter* Sp*	*Virgibacillus pantothenticus***
Morphological characters			Indole production	-	-
Colony color	White	Yellow	Acetoin production	+	+
Colony margin	Smooth	irregular	Gelatinase production	-	+
Colony elevation	Flat	Low convex	Oxidase production	+	+
Colony configuration	Rod shape	rod shape	Catalase production	+	+
Gram reaction	-	+	Nitrate production	+	+
Presence of spores	-	+	Utilization of		
Physiological characters			D-glucose	-	+
β-galactosidase production	-	+	D-mannitol	-	-
Arginine dihydrolase	+	-	Inositol	-	-
Lysine decarboxylase	-	-	D-sorbitol	-	-
Ornithine decarboxylase	+	-	D-rhamnose	-	-
Citrate utilization	+	+	D-sucrose	-	+
H2S production	-	-	D-melibiose	-	-
Urease production	-	+	Amygdalin	+	-
Tryptophane deaminase	-	-	L-arabinose	-	-

*Achromobacter sp. HEGN 014, **_Virgibacillus pantothenticus_ HEGN 114.

Table 2. Resistance of thermo-tolerant strains to the different antibiotics.

Tested antibiotics	*Achromobacter* sp. HEGN 014	*Virgibacillus pantothenticus* HEGN 114
Ampicillin/sulbactam, 20 µg	-*	+
Ciprocin, 5 µg	-*	+
Gentamycin, 10 µg	+	+
Ampicillin,10 µg	+	+
Imipenem, 10 µg	+	+
Norfloxacin, 10 µg	+	+
Cephalexin, 30 µg	+	+
Cefadroxil, 30 µg	+	+
Ceftazidine,30 µg	+	+
Erythromycin, 15 µg	+	+
Flucloxacillin, 5 µg	+	+

*Detection of inhibition zones, 20 and 30 mm in the presence of Ampicillin/sulbactam, 20 µg and Ciprocin, 5 µg.

Table 3. Production of different enzymes by the selected strains.

Tested enzymes	*Achromobacter* sp HEGN 014	*Virgibacillus pantothenticus* HEGN 114
Gelatinase	-	+
protease	-	-
Lipase	++	+
Chitinase	-	-
Cellulase	-	-

followed by anion exchanger chromatography step for getting the purified lipase and the purity of the enzyme was confirmed by Gel filtration using Sephadex G-100 (data not shown).

For selection of optimum temperature for the highest activity of purified lipase, the reaction temperatures

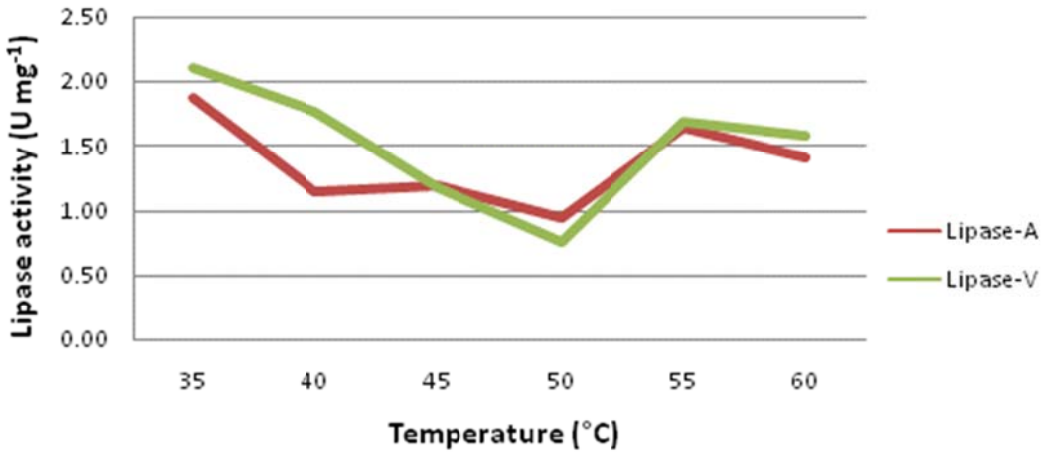

Figure 7. Effect of different temperatures on lipase activity. Lipase-A = lipase produced by *Achromobacter* sp. HEGN 014, Lipase-V = lipase produced by *V. pantothenticus* HEGN 114.

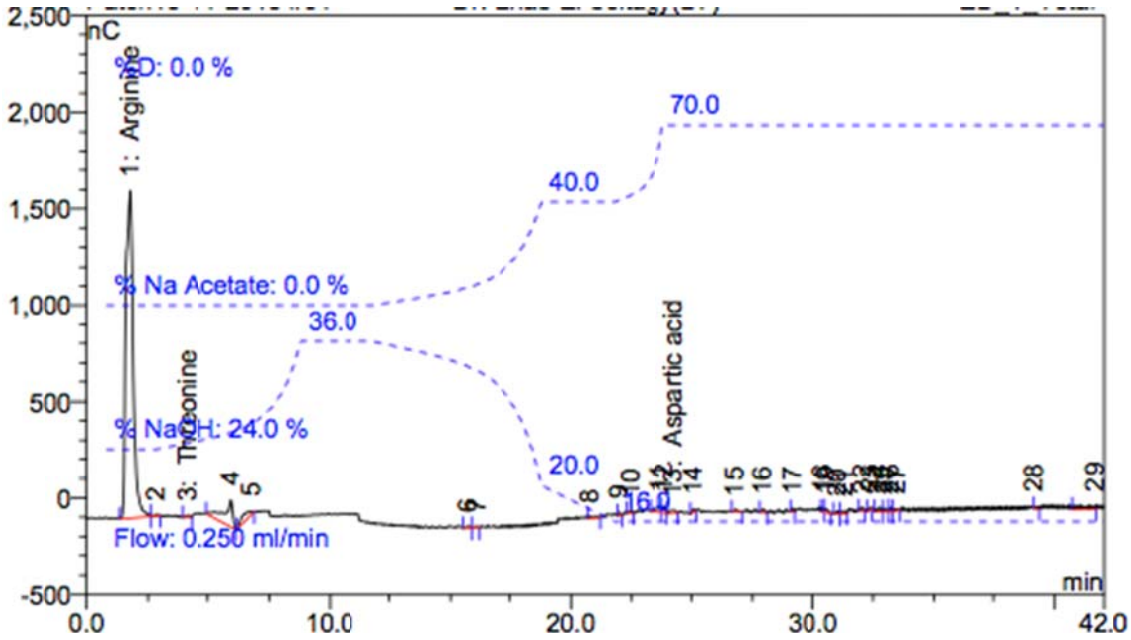

Figure 8. Amino acids analysis of purified lipase from *Achromobacter* sp. HEGN 014.

varying from 35 to 60°C were selected and examined for both purified lipase of *Achromobacter* sp. HEGN 014 and *V. pantothenticus* HEGN 114. The data in Figure 7 shows that, the purified lipases (lipase-A and lipase-V) produced from *Achromobacter* sp. HEGN 014 and *V. pantothenticus* HEGN 114 had the same pattern with slight difference in lipases activity. The optimum reaction temperature giving the highest activity for lipase-A and lipase-V was 35°C recording 1.88 and 2.11 U mg^{-1}, respectively. The enzymes activities of lipase-A and lipase-V at 50°C decreased to remain 52 (1.64 U mg^{-1}) and 36% (1.69 U mg^{-1}), respectively, of initial activity at 35°C. Yet, both lipase-A and lipase-V moved upward to increase the lipases activity to reach temperature of 55°C recording 91 and 80% of initial lipases activity at 35°C.

Amino acid analysis

The amino acid analysis (Figure 8) of the purified lipase produced from strain *Achromobacter* sp. HEGN 014 showed the presence of 3 detectable peaks of amino acids. These amino acids were detected as arginine, threonine and aspartic acid at peak no. 1, 3 and 13.

Arginine was the major one representing about 99.26% of the total amount of detected amino acids (90.651 mmole) as the amount of arginine was estimated to represent about 89.982 mmole. Unlike arginine, threonine and aspartic acid showed the lowest percentages (0.21 and 0.53%), respectively, of total detected amino acids in the purified lipase. The amount of threonine and aspartic acid were 0.198 and 0.480 mmole, respectively.

DISCUSSION

A major impetus that has driven extensive and intensive research efforts on extremophiles during the last decades is the potential biotechnological applications associated with these microbes and their products. The likely potential has been increasing exponentially with the isolation of new microbial strains, the identification of novel compounds and pathways, and the molecular and biochemical characterization of cellular components (Satyanarayana et al., 2005).

Thermo-tolerant microorganisms are the organisms belonging to the mesophilis, but which adapt to live in a higher temperature environment (Suntornsuk et al., 2005). Although thermophilic microorganisms usually produce thermostable enzymes, limited mesophilic microorganisms can also produce thermostable or thermotolerant lipases. Microbial extracellular enzymes are of considerable commercial interest for biotechnological applications as they can be produced at low cost (Khoramnia et al., 2011).

The results of this work showed that North Delta sediments in Egypt are a rich source of thermo-tolerant bacteria, which could be a good source of many interested enzymes from the industrial point of view and further studies are needed on this area including study of microbial biodiversity and the biotechnological applications of the isolated strains. It was found that the occurrence of thermo-tolerant bacteria in North Delta sediments at 55°C reached 11.1% with respect to the total viable count at 30°C.

The isolates were either alkalitolerant (showed very good to excellent growth from pH 7.0 to 9.0, but no growth at pH 11) or alkalophilic (showed very good and excellent growth between pH 7.0 and 11.0), while they show less cellular yield at pH.6.0 (Khalil, 2011). In North Delta sediments, five from the seven (71.4%) selected bacterial isolates showed optimum growth at pH 9.

According to the definition of halotolerant microorganisms, they are identified as microorganisms that have no specific requirement for salt other than the usual NaCl needed by all (non-halotolerant) organisms (Khalil, 2011). Halophiles can be classified into three groups on the basis of their response to NaCl, slightly halophiles which grow optimally at 2-5% (0.2-0.85 M), moderate 20% NaCl (0.85-3.4 M) and the extreme halophiles which optimally grow at 20-30% NaCl (3.4-5.1 M) (Jayachandra et al., 2012). In the present study, all bacterial isolates gave the maximum growth at 4% (w/v) NaCl. Two thermo-tolerant bacterial isolates from North Delta sediments (Egypt) were identified as *Achromobacter* sp. HEGN 014 and *Virgibacillus pantothenticus* HEGN 114.

The species *V. pantothenticus* HEGN 114 and related organisms comprising this new genus can be distinguished from members of *Bacillus* rRNA group 1 (*Bacillus sensustricto),* and from members of *paenibacillus* and other aerobic endospore-forming bacteria by routine phenotypic tests (Heyndrickx et al., 1998). Subsequently, four further species, *Virgibacillus proomii*, *Virgibacillus carmonensis*, *Virgibacillus necropolis* and *Virgibacillus picturae*, were described. *Virgibacillu*s are very abundant in the coastal regions of Karwar and Mangalore, due to its potentiality in producing the extracellular hydrolytic enzymes; it has gained importance in industries for their commercial usage (Rohban et al., 2009; Jayachandra et al., 2012).

Some extremophilic microorganisms are able to overcome more than one type of extreme conditions in their environment (Oren, 2008). The optimum growth of thermo-tolerant *Achromobacter* sp. HEGN 014 and *V. pantothenticus* HEGN 114 isolated from North Delta sediments were at pH 9, so it is considered as alkalophilic, and also tolerate concentrations of NaCl reached 7 and 9% (w/v), respectively.

An extremely halophilic bacterium *Virgibacillus* sp. strain JS5 was isolated from the Arabal soil of west coast of Karnataka, India. The strain was Gram positive, motile rod shaped cells and spore forming. It was strictly aerobic, ferments several carbohydrates. Catalase and oxidase test were found to be positive. Also, the strain grew in the presence of 0-25% (w/v) NaCl, with optimum growth at 10% (w/v) NaCl, pH of 6-10 and temperature range of 20-45°C, with an optimum growth temperature of 35°C, showing that the halophilic bacterium belong to the extremophilic group. It has potential to produce the extracellular enzymes such as amylase, protease, inulinase and gelatinase (Jayachandra et al., 2012, 2013).

Gray et al. (2010) mentioned that *Achromobacter* is a genus containing members that are Gram negative and oxidase positive. They are rod shaped, have flagella and grow well at 37-42°C with a pH 6.5-8.5. Also, *Achromobacter* sp*.* is widespread in aquatic habitats (Coenye et al., 2003).

The thermo-tolerant *Achromobacter* sp. HEGN 014 and *V. pantothenticus* HEGN 114 isolated from North delta sediments were antibiotics resistance. Duggan et al. (1996) reported that most *Achromobacter* species isolates have been found to be resistant to first- and second-generation cephalosporins, aminoglycosides and narrow-spectrum penicillins; susceptible to sulfonamides, carbapenems, broad-spectrum penicillins and third-generation cephalosporins; and variably susceptible to fluoroquinolones, ceftazidime, piperacillin, imipenem,

ticarcillin/clavulanic acid and varying degrees of resistance to ciprofloxacin and of loxacin.

The extracellular bacterial lipases are of considerable commercial importance, as their bulk production is much easier. Although, a number of lipase-producing bacterial sources are available, only a few are commercially exploited as wild or recombinant strains. Of these, the important ones are: *Achromobacter, Alcaligenes, Arthrobacter, Bacillus, Chromobacterium and Pseudomonas* (Gupta et al., 2004)

In fact, it is generally true that the enzymes of an organism are adapted to function optimally at or near its growth conditions, accordingly, the range of extremes at which life is found defines the range of conditions at which enzyme activity might be detected. In particular, it is believed that the outstanding stability of extremophilic enzymes will contribute to filling the gap between chemical and biological processes (Khalil, 2011).

Potential enzymes such as amylase, protease, DNA polymerase, xylanases and chitinases have been identified in thermophilic microorganisms including the lipase enzyme (Zuridah et al., 2011; Tayyab et al., 2011). The lipases used are usually of fungal or bacterial origin (Kanwar et al., 2006). Bacterial lipases are mostly extracellular and are greatly influenced by nutritional and physico-chemical factors, such as temperature, pH, nitrogen and carbon sources, inorganic salts, agitation and dissolved oxygen concentration (Gupta et al., 2004; Mobarak-Qamsari et al., 2011; Padhiar et al., 2012).

In the present study, the thermo-tolerant *Achromobacter* sp. HEGN 014 and *V. pantothenticus* HEGN 114 produced an extracellular lipase, presumably because the activity was associated with the cell (Hande-lsman and Shoham, 1994). Some thermophilic microbial strains are able to produce thermostable lipases (Stathopoulou et al., 2013).

The optimum reaction temperatures giving highest activity of the purified lipases from *Achromobacter* sp. HEGN 014 and *V. pantothenticus* HEGN 114 were obtained at 35°C followed by a few lost in activity at 55°C, and the enzyme showed a relatively low activity in a temperature ranging from 40 to 50°C. The most likely explanation for this result is that the lipases could exist in isozyme form as multiform of lipase with the same function in terms of lipase activity.

Chahinian et al. (2000) found that lipase enzyme produced from *Pennicllium cyclopoium* exists in several glycosylated forms (40-43 KDa). This result shows that one form of this isozyme is a thermo-tolerant lipase which tolerate the reaction temperature of 55°C and the other one is non-thermo-tolerant lipase where the optimum temperature was 35°C. This temperature range agreed with other lipases purified from other microorganisms. Lee et al. (1999) found that the optimum temperature for thermophilic *Bacillus thermoleovorans* ID-1 was between 70-75°C. Also, Kaminishi et al. (1999) found that the optimum temperature for *Eurotrium hebariorum* NU-2 was 37°C. In addition, the optimum temperature for *Pichiaburtonii* was found to be 45°C (Sugihara et al., 1995) and for *Pseudomonas sp*. was between 45-60°C (Dong et al., 1999).

Amino acids analysis of lipase from *Achromobacter* sp. HEGN 014 proved to be rich in arginine. This result indicates that arginine plays a vital role in the mode of action of lipases with the substrate especially at higher temperature. The most likely explanation for the other undetected amino acids is that the concentrations of other amino acids were very low due to low concentration of the sample as compared to the higher concentrations of arginine, threonine and aspartic acid in *Achromobacter* sp. HEGN 014. A few research papers have discussed the role of arginine in simulating the release of lipase (Morita et al., 2008).

Conflict of interests

The authors did not declare any conflict of interest.

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